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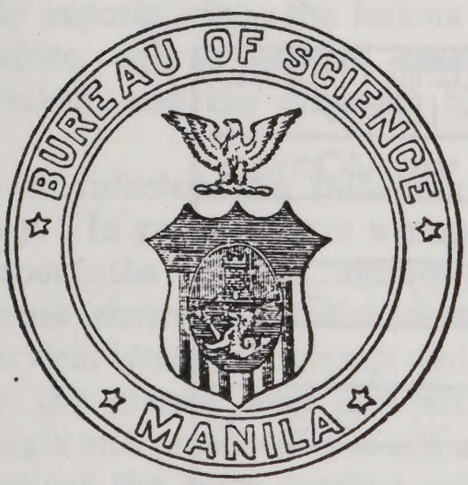

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STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

IV. PORTAL OF ENTRY OF INFECTION AND METHOD OF DEVELOPMENT OF THE LESIONS IN PNEUMONIC AND PRIMARY SEPTICÆMIC PLAGUE: EXPERIMENTAL PATHOLOGY.

By Richard P. Strong and Oscar Teague.

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

For the purpose of studying experimentally the question of the portal of entry of the organism and the method of the development of the lesions in pneumonic plague, animals were placed in closed glass cages, and agar-cultures of virulent pneumonic strains of the plague bacillus suspended in saline solution were sprayed for a period of from about two to three minutes into the surrounding air which they breathed. Thirty-four normal guinea pigs and 55 normal monkeys were so infected with plague bacilli, and all succumbed to plague infection. The animals were necropsied in each instance, and the lesions present observed and studied. It would be very tedious to record here the individual necropsy reports, since the lesions found were so often similar. Therefore, only a general description of the lesions will be undertaken, and the different types of lesions emphasized.

In the guinea pigs so infected, the following changes were encountered at necropsy. In general there was marked evidence of plague infection about the cervical and laryngeal tissues. The subcutaneous tissues showed extensive œdema, and there was swelling of the cervical lymphatic glands and of those about the trachea. Usually the glands were not only swollen but more or less hæmorrhagic and presented the appearance of small early buboes. Throughout the body marked evidences of septicæmia were usually present. There were frequently extensive hæmorrhages in the intestinal wall. The spleen sometimes showed the typical changes encountered in bubonic-plague in-

fection with miliary abscesses. Distinct evidences of pneumonia were present in only about 23 per cent of the guinea pigs. Plague bacilli were frequently not very abundant in the lungs, unless pneumonic areas were encountered, but were always present in the heart's blood. The lungs were sometimes reddened, congested, and œdematous, and sometimes contained hæmorrhagic infarcts. Small areas of primary bronchial pneumonia were encountered in some of the cases, and in one a whole lobe of the lung showed pneumonic engorgement. In two instances either red or early gray hepatization was present. Numerous miliary abscesses were occasionally encountered in the lungs. (See Plate VII.) The areas of bronchial pneumonia were firm, contained no air, and were usually irregular in outline and red, reddish yellow, or yellow in color. On cut section they were sometimes wedge-shaped. In those instances in which hæmorrhagic infarcts, miliary abscesses, and in addition reddish-yellow or yellow areas of lobular pneumonia are present (see Plate VII), we must conclude that the infection of the lung is secondary, and that in these instances we are not dealing with primary pneumonic plague, in which infection enters through the bronchi, but with secondary infection of hæmotogenous origin. Such a conclusion is supported by the microscopical study of these lesions. A section of the lung in the vicinity of one of the hepatized areas, pictured in Plate VII, shows the bacteria in very large numbers both about and within the small blood vessels, and in places infarctions have occurred; numerous hæmorrhages from the vessels have also taken place; in the neighborhood of the pneumonic areas the bacteria are also plentiful in the lung alveoli and in the perivascular spaces.

Therefore, these changes suggest that the primary point of infection did not always occur in the bronchi or alveoli of the lung. From a study of all the lesions in guinea pigs, it would appear that these animals, under the conditions of the experiments in which the spraying was carried on, did not frequently develop primary plague pneumonia, but that infection occurred through the mucous membranes of the mouth and throat, resulting in a general septicæmia generally preceded by the formation of early buboes of the cervical glands and sometimes followed by the development of secondary areas of plague pneumonia. It would appear that in guinea pigs, either on account of too shallow respiration or the small size of the larynx and trachea, the bacteria are not so likely to penetrate to the smaller bronchi by means of the inspired air. Instead, they are apparently

deposited largely upon the mucous membranes of the mouth and throat.

The experiments performed on monkeys seem to throw much more light upon the mode of pneumonic-plague infection in man. The lesions in 55 monkeys infected by spraying were studied at necropsy. There was a marked similarity in general in the pathological changes encountered. In practically all of the animals there was absence of any sign of plague infection about the cervical tissues. The submaxillary and cervical lymphatic glands and those about the trachea were not swollen, nor was there any œdema of the cervical tissues, as was practically always seen in the experiments with guinea pigs. In none of the cases examined did the tonsils show evidence of primary disease, though in a number of instances they were sectioned and stained. In some instances they were moderately congested. Plague bacilli were scanty in them and when present were not more numerous than in the heart's blood and never so numerous as they were in the lungs or spleen.

There was frequently œdematous fluid in the trachea, and in a few cases the trachea was slightly reddened. The larynx and vocal cords were not as a rule injected. There was not such marked evidence of septicæmia as seen in the experiments with guinea pigs, but plague bacilli could always be recovered from the heart's blood by culture. No hæmorrhages were noted in the intestines and omentum. The spleen and liver showed no miliary abscesses. There were no cervical, axillary, nor inguinal buboes. The lungs showed primary pneumonic changes in every case. There was always much œdema. In those animals which succumbed a shorter time after infection, the lobular type of pneumonia was much more frequently encountered. In those which survived a longer period, whole lobes of the lung usually showed pneumonia. The progress of the lesions is well shown in Plate VII, figs. 2 and 3. The process evidently begins as a lobular bronchial pneumonia. By the fusion of a number of the areas of lobular pneumonia, the whole lung may become involved. The large pneumonic areas were either in the stage of engorgement or of red or early gray hepatization. In a number of cases a pleuritic exudate was observed over the hepatized areas. In no case were miliary abscesses observed in the lungs. In the cases with the *early lesions*, the plague bacilli were always most numerous in the lungs, and in section were found in greatest profusion about the bronchioles, in the peribronchial lymph spaces and alveoli, and beneath the pleura. In some instances the cells

lining the alveoli appear normal even when they contain large numbers of bacilli. Although the blood vessels between the lobules and septa were dilated, and hæmorrhages sometimes occurred, practically no bacteria were found within them.

From these observations, it is obvious that the infection in monkeys occurred by inhalation and resulted in primary plague pneumonia.

It also is evident that in some instances in which monkeys are exposed to infection by inhalation, the primary point of infection may be not only the lungs, but also the mucous membranes of the mouth and throat. That plague infection may occur through the mucous membranes of the mouth and throat *alone* in monkeys was demonstrated by placing a small quantity of plague bacilli upon the posterior portion of the throat by means of a glass rod. The following experiments are given as examples of such infection.

EXPERIMENT I.

Monkeys Nos. 5882, 5883, and 5884 were all infected in the following manner on November 7.

A necropsy was performed upon monkey No. 5876 which had just died of experimental pneumonic plague and a portion of the pneumonic lung was cut into small pieces in a Petri dish. A glass rod with the end rounded in a flame was dipped into the œdematous fluid in the Petri dish and passed over the tongue and rubbed against the pharynx of each of the three monkeys (Nos. 5882, 5883, 5884). The monkeys held their tongues so that the glass rod was squeezed between the soft palate and the tongue and most of the material on the rod was evidently caught there. All three of the monkeys were treated in the same way and then the rod was dipped into the same fluid and touched to the shaved skin of a guinea pig as a control. The control guinea pig, No. 5885, died November 14, seven days after, with typical lesions of plague.

Monkey No. 5882 was found dead on Nov. 13, six days after infection. *Necropsy*: The superficial cervical glands are swollen on both sides. Both submaxillary glands are swollen and hæmorrhagic. The changes are more marked on the left side. The deep cervical glands on both sides are also swollen and hæmorrhagic; the process is more advanced on the left side. The axillary lymph nodes are also swollen and hæmorrhagic. The lesions in these glands are more marked on the right side. The tonsils on both sides are swollen and reddened. The larynx shows slight injection. The trachea contains a small amount of pale, frothy fluid; its mucosa is not injected. The bronchial lymph nodes at the bifurcation of the trachea are very small. There is no evidence of pneumonia in either lung. The spleen is much swollen and very soft. Smears from the spleen and blood show innumerable plague bacilli. Smears from the cervical and axillary glands show very numerous plague bacilli. Smears from the lung show fair numbers of plague bacilli. Sections of the tonsils show no evidence of primary plague infection and but few bacilli.

Monkey No. 5883 was found dead on November 15, eight days after infection. *Necropsy:* The tonsils are pale; they contain few pest bacilli and many cocci. The submaxillary, deep cervical, and axillary glands are small and deep red in color. A gland at the bifurcation of the bronchi is enlarged and reddish black in color and contains many plague bacilli. The pharynx and larynx are slightly reddened. The trachea contains some pale, frothy fluid. The lungs are pale and show no pneumonic areas. Cultures made. The spleen is enlarged, deep red, firm, and contains large numbers of plague bacilli. The blood also contains large numbers of plague bacilli.

Guinea pig No. 5902 inoculated with the spleen of monkey No. 5883. Died in four days with large numbers of plague bacilli in its spleen and with well-marked buboes.

Guinea pig No. 5901 inoculated with the lung of monkey numbered 5883. Died in seven days with large numbers of plague bacilli in its spleen and with well-developed buboes.

Monkey No. 5884 was found dead on November 14, seven days after infection. *Necropsy:* (By Dr. Crowell.) The tonsils and pharynx are considerably reddened and covered with frothy fluid. The tonsils are small and pale, show no hæmorrhages, and are probably not enlarged. The submaxillary glands are slightly enlarged and deep red. The deep cervical glands are somewhat enlarged and deep red, redder than the submaxillary. The glands in both axillæ are enlarged and hæmorrhagic, those in the left being larger than in the right. A gland at the bifurcation of the trachea is small, but deep red in color. The larynx and trachea are slightly reddened throughout their extent and contain abundant frothy, slightly blood-tinged fluid. The lungs show on the surface only a few small red areas. No pneumonia is present. On section, the cut surface is dark red and very moist. The spleen is enlarged and fairly firm.

EXPERIMENT II.

Culture No. 32 isolated from a pneumonic-plague case at Mukden was passed through a series of guinea pigs and a fresh culture from one of these passage guinea pigs was suspended in saline solution. A glass rod was dipped into this suspension and touched against the pharynx of three monkeys (Nos. 5927, 5928, 5929) as in the preceding experiment. The following necropsy reports show that the bacilli from artificial cultures brought about the same result as those inoculated directly from the pneumonic lung.

Monkey No. 5927.—Bacillus pestis placed on mucosa of mouth December 5. Found dead six days later. *Necropsy:* (By Dr. Crowell.) The axillary glands in the right axilla are slightly reddened, in the left they are pale. The tonsils show no visible change. There is no pneumonia present. The spleen is somewhat enlarged. Smears from the heart show numerous bipolar organisms. The spleen contains involution forms. The liver contains numerous bipolar organisms which are less numerous in the lungs than in the blood.

Monkey No. 5928.—Bacillus pestis placed on mucosa of mouth December 5. The animal died December 14. *Necropsy:* (By Dr. Crowell.) The tonsils, deep cervical, submaxillary, and axillary glands are only slightly swollen and reddened. The lungs are somewhat œdematous and a little

reddened. There is no consolidation. The trachea is only slightly reddened. The spleen slightly, if at all, enlarged. Smears from the heart's blood show very numerous pest bacilli, while the organisms in the lung, liver, and spleen are numerous.

Monkey No. 5929.—Inoculated on December 5, died on December 10. *Necropsy:* (By Dr. Crowell.) Smears from the spleen show very few, if any, pest bacilli. The heart shows two or three to a field. Few are found in the lung, while the liver shows numerous bacilli. The lesions are practically the same as those encountered in monkey No. 5928.

Therefore, these animals all died of plague septicæmia with or without bubonic infection of the cervical glands; that is, in the case in which the infection was severe and the susceptibility of the animals more marked, they succumbed to septicæmia before cervical buboes developed. In none of these instances was pneumonia present. Primary plague pneumonia only results when infection by inhalation has in addition taken place.

It has been claimed by several observers and more recently by Koulecha [1] that pneumonic plague in man is primarily a septicæmic disease, the lungs becoming secondarily involved by way of the blood circulation. According to this observer, the infection is supposed to spread from the perivascular spaces to the neighboring lung alveoli. He further believes that the bacilli enter the blood by the lymph vessels through the lesions in the tonsils and are deposited in the interstitial tissues around the lung alveoli, the tonsils being regarded as the primary point of infection. In some instances he assumes it to be possible for the plague bacilli to pass from the mucous membranes of the trachea and bronchi to the neighboring lymphatic glands and from them to enter the blood and in this way later to reach the lung. Albrecht and Ghon have shown that by the intravenous injection of plague bacilli in animals, pneumonic plague did not result.

In our opinion, the view that pneumonic plague is primarily a septicæmic disease and that the lungs become secondarily involved by way of the blood circulation and that the tonsil is first infected is not acceptable.

From our study of pneumonic plague both in man [2] and animals, we feel justified in concluding that infection in epidemic pneumonic plague results from inhalation, the primary point of

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 154.

[2] For a description of the human lesions, see VII, p. 210 of this report.

infection being not in the tonsils[3] but some portion of the bronchi, the organism either passing along the bronchioles directly to the alveoli or through the walls of the bronchioles to the contiguous tissue of the lungs, giving rise, first, to peribronchial and perivascular inflammation in the surrounding tissues, and then to more diffuse inflammatory processes throughout the lung. Having reached the lung tissue, the bacilli rapidly multiply and produce at first pneumonic changes of the lobular type and shortly afterward more general lobar involvement of the lung tissue.[4]

The blood becomes quickly infected and a true bacteræmia results in every case. The fact that the bronchial glands at the bifurcation of the trachea are always much more severely affected than any of the other lymphatic glands also argues against the theory that epidemic pneumonic plague is primarily a septicæmic disease and that the lungs are infected secondarily from the blood. Moreover, in the earliest stage of the disease, the blood may be free from plague bacilli as we have shown by cultures.

It is true that in some instances the bacteræmia occurs early in the course of the disease and before hepatization of the lung may have taken place. However, microscopical examination will reveal enormous numbers of plague bacilli in the engorged lung tissue from which it appears that the origin of the bacteræmia is clear.

The tonsils may become secondarily infected in pneumonic plague just as other lymphatic glands—for example, the bronchial ones—become so infected. However, in pneumonic plague death usually occurs before any marked macroscopic changes occur in the tonsils. There is no doubt also that the tonsils may become primarily infected in epidemics of pneumonic plague just as has occurred in sporadic cases in epidemics of bubonic plague; such cases have been previously reported. This, however, is not the common channel of primary infection, and in such cases involvement of the glands of the neck occurs early in the course of the disease. Such cases are really instances of bubonic plague in which the lungs may, or may not, become secondarily infected.

In some instances plague infection may occur directly through

[3] See also under pathological anatomy, p. 215 of this report for the condition of the tonsils in the human cases and Plates XI and XVIII.

[4] See Plate VII, figs. 2 and 3, and Plates IX and X.

the mucous membranes of the mouth and throat. Primary septicæmia may then result. In those instances in which the infection is virulent and severe and the susceptibility of the host marked, death may sometimes occur before bubonic involvement is apparent. In other instances, bubonic involvement of the glands of the neck and septicæmia are present. No true pneumonia occurs unless infection by inhalation has in addition taken place. The German and the Austrian Plague Commissions concluded that primary plague septicæmia probably does not exist. However, these commissions made their observations only during epidemics of bubonic plague. From our studies made upon human beings, during the Manchurian epidemic, as well as from the animal experiments quoted above, we must conclude that primary plague septicæmia does sometimes take place and that death may occur, though rarely, before visible lesions have taken place either in the lungs or lymphatic glands.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

V. CLINICAL OBSERVATIONS.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

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SYMPTOMS.
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TYPES OF THE DISEASE MET WITH DURING THE MANCHURIAN EPIDEMIC.

The cases throughout the epidemic were almost entirely those of primary pneumonic plague, only two or three undoubted cases of primary bubonic infection having been reported at the International Plague Conference.[1] However, in a number of instances death occurred before there were any clinical manifestations that pneumonic plague was present, and in some of these cases only at necropsy was it discovered that early involvement of the lungs existed. This led to the belief that many of the cases were primarily septicæmic in character. One observer at the Conference, Doctor Christie,[2] estimated from a clinical standpoint that about 5 per cent of the cases was of the septicæmic variety without pneumonia. However, from the post-mortem studies made during the epidemic, we must conclude that the cases with no involvement of the lungs were exceptional ones. Nevertheless, in a few instances in which infection did not occur by inhalation but probably through the tonsils or the mucous membranes of the mouth or throat, it seems unquestionable that the lungs were either not involved or only very slightly so. Thus,

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 428.

[2] *Ibid.*, p. 166.

in one instance of this nature, occurring during the epidemic and reported by Fujinami,[3] the lymphatic glands of the neck showed enlargement with hæmorrhages, and the surrounding tissues of the pharynx and larynx were very much affected, while the lung was only very slightly involved. Obviously, this case should be regarded as primarily of bubonic character. Both the German and the Austrian plague commissions concluded that primary plague septicæmia probably does not exist. However, as has already been called attention to elsewhere in this report, these commissions made their observations only during epidemics of bubonic plague. From the studies made upon human beings during the Manchurian epidemic, as well as from the animal experiments performed in this laboratory,[4] we must conclude that primary plague septicæmia does sometimes occur, death resulting from this cause before lesions, which are macroscopically recognizable, are present in the lymphatic glands or in the lungs.

Several cases of primary intestinal plague were reported at the Conference in which bloody diarrhœa appeared to be the most prominent symptom. None of these cases was studied at necropsy. It appears that no definite evidence of the occurrence of primary intestinal infection during the epidemic was produced. In the few instances in which plague bacilli had been found during the epidemic in the fæces, infection had evidently occurred secondarily from the blood. Albrecht and Ghon in the report of the Austrian Commission have reported the only suggestive case of primary intestinal plague occurring during a bubonic epidemic of plague, and even in this case the evidence of such infection is not conclusive. However, it seems established that primary intestinal plague has been produced in rats by feeding large quantities of virulent plague bacilli. In many instances during the Manchurian epidemic, the patients with pneumonic plague must have swallowed enormous numbers of plague bacilli in the saliva and sputum. Nevertheless, in none of the necropsies performed during the epidemic were evidences of primary intestinal infection present nor was serious involvement of the intestine encountered. This fact certainly speaks strongly against the existence of primary intestinal plague in man and would seem to show that even if the intestines are sometimes secondarily involved, this condition in human beings must be also a very rare one.

[3] *Ibid.*, p. 150.

[4] See IV, p. 173 of this report.

SEX, AGE, AND INCUBATION PERIOD.

Both sexes seem equally susceptible, but the proportion of females and children attacked during the epidemic was comparatively small, as women and children were evidently not so frequently exposed to infection. The disease prevailed particularly among the poorer classes, coolies, etc., the majority of whom were between 20 and 40 years of age. The incubation period varied from two to five days, though usually it was not over two or three days.

SYMPTOMS.

The following summary of the clinical features of the disease has been made largely from personal observations during the epidemic in Mukden and also from evidence presented at the International Plague Conference.

The onset of the disease is usually somewhat abrupt; prodromal symptoms are rare. The disease usually begins with chilly sensations, but a distinct rigor generally does not occur. Epistaxis is generally not present. There is headache, loss of appetite, an increase in the pulse rate, and fever. Vomiting rarely occurs. Within from twenty-four to thirty-six hours after the onset, the temperature usually has reached 103° or 104° F., and the pulse 110 to 130 or more beats per minute. Cough and dyspnœa usually appear within twenty-four hours after the onset of the first symptoms. The cough is usually not painful. The expectoration is at first scanty, but soon becomes more abundant. The sputum at first consists of mucus which shortly becomes blood-tinged. Later the sputum becomes much thinner and of a bright-red color; it then contains enormous numbers of plague bacilli in almost pure culture. The typical rusty sputum of croupous pneumonia has not been observed. The conjunctivæ become injected, and the tongue coated with either a white or brownish layer. The expression is usually anxious, and the face frequently assumes a dusky hue. Labial herpes has never been observed. The patients sometimes complain of pain in the chest, but usually this is not severe. Apart from the disturbances due to the dyspnœa and their anxiety for their condition, they usually appear to suffer but little and usually do not complain of pain. In the later stages of the disease, the respirations become greatly increased and the dyspnœa usually very marked, the patients frequently gasping for air for several hours before death. Cyanosis is then common.

The signs of cardiac involvement are always marked in the

advanced cases, the pulse becoming gradually more rapid, feeble, and running; finally it can not be felt.

Gallop rhythm of the heart sounds are frequently observed. Death takes place from cardiac paralysis and exhaustion. The patients frequently succumb after slight physical exertion, such as sitting up in bed to take nourishment or on being moved. A few hours before death the temperature often declines to below normal. Delirium and coma are frequently present before death.

The urine in the later stages may show the presence of albumin. The diazo and indican reactions have not been observed in the few cases in which the urine was tested. Extravasations of blood have been found in the pelves of the kidneys at post-mortem examination.

The spleen is usually not palpable, and the lymphatic glands not enlarged. Petechiæ or larger hæmorrhages of the skin are usually not present. Bloodly diarrhœa is occasionally observed. Plague bacilli frequently may be present in the blood in such numbers that a simple, microscopical examination suffices for their detection; in other cases, cultures are necessary for their discovery. A marked leucocytosis may occur, though in some cases the leucocytes are not increased. In the *primary septicæmic* cases the course of the disease is very rapid. There may be no manifestations of disturbances of the lung. The cardiac symptoms are very prominent. The patients soon pass into a comatose condition and die.

PHYSICAL SIGNS.

The physical signs in the lungs are often slight, even in cases well advanced in the disease. On percussion, dulness is often absent, and the vocal fremitus and resonance unchanged. In a small proportion of cases, however, smaller or larger areas of dulness may be discovered. On auscultation râles are frequently not present, except shortly before death. When present early in the disease, they are usually of the fine variety. Numerous moist râles are heard late in the disease, and are due to the œdematous condition of the lungs. The character of the râles is in accordance with what one would expect from the condition of the lungs and bronchi and the character of the exudate observed at necropsy. Coarse râles such as occur in cases of catarrhal bronchitis usually are not present. Feeble, respiratory sounds, tubular modification, or pure tubular respiration over small areas are the conditions found most commonly on auscultation. Not infrequently a dry, pleuritic rub is heard.

The limits of dulness of the heart are sometimes increased

to the right of the sternum. The heart sounds are rapid and usually become feeble or embryocardiac in character toward the end. In the early stages the secondary pulmonic sound may be accentuated, but it soon becomes much less distinct.

DIAGNOSIS.

The diagnosis is usually clear from the bacteriological examination of the sputum in which the bacillus is found in enormous numbers and in almost pure culture. A rise in temperature and an increased pulse rate are usually the earliest symptoms observable, but before the sputum appears the diagnosis may be doubtful. An examination of the blood, either microscopically or by culture, may reveal the diagnosis, since during the past epidemic all the cases became septicæmic. The blood should always be examined early, by cultural methods, as in the primary septicæmic cases involvement of the lungs may not occur. The bacteriological diagnosis is the only certain one for excluding pneumonic infection due to microörganisms other than *Bacillus pestis*, but from the general condition of the patient, in connection with the absence of marked physical signs in the lungs, the diagnosis of pneumonic-plague infection is often particularly suggested. Labial herpes has not been observed in primary pneumonic plague. The presence of numerous coarse, piping or sibilant bronchial râles in the lungs is an argument against pneumonic-plague infection. The sputum in pneumonic plague is not purulent as it frequently is in catarrhal bronchitis or in bronchial pneumonia, and it is not so tenacious and has not the rusty appearance of the sputum so often seen in croupous pneumonia. The cough is usually not so painful as in croupous pneumonia.

The duration of the disease is usually less than two days, though many cases did not live longer than sixteen hours after the onset of symptoms. Cases sometimes survived for three, and, more rarely, for four days. In no case reported was the duration over one week.

PROGNOSIS AND TREATMENT.

The prognosis is unfavorable. No cases in which the bacteriological diagnosis was complete were known to have recovered during the Manchurian epidemic.

No method of treatment appeared in any way to have been successful. Treatment with serum seemed, in a few instances, to have prolonged the duration of the illness.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

VI. BACTERIOLOGY.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

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CHARACTERS OF THE PNEUMONIC STRAIN OF "BACILLUS PESTIS."
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INFECTIVITY OF THE EXCRETA OF THE PLAGUE PATIENT.

BACTERIOLOGICAL DIAGNOSIS OF PNEUMONIC PLAGUE FROM:
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CHARACTERS OF THE PNEUMONIC STRAIN OF "BACILLUS PESTIS."

During the epidemic in Manchuria the idea became rather general that the organism of pneumonic plague differed, in some respects at least, from *Bacillus pestis* of bubonic plague. Apart from cultural variations, some physicians believed that while the bacillus of bubonic plague on inoculation into guinea pigs gave rise to buboes, the bacillus of this epidemic, on injection into these animals, caused only pneumonia and septicæmia. Also, it was claimed by some, that the virulence of the organism of pneumonic plague was much greater than that of the bacillus of bubonic plague. These ideas were erroneous, as is apparent from a consideration in detail of the properties of the pneumonic strain arrived at from the study of numerous microscopical preparations and cultures obtained from the sputum and from necropsies performed during the epidemic.

MORPHOLOGY.

From a morphological standpoint, the causative organism of the Manchurian epidemic of pneumonic plague apparently differs in no respect from other strains of *Bacillus pestis* isolated during

many epidemics of bubonic plague. In stained microscopic preparations made from the organs at necropsy it appears in its most characteristic form as a short bacillus, more or less ovoid in form, swollen in the center, and rounded at the ends. It exhibits marked bipolar staining, the central portion either remaining uncolored or staining lightly. Such preparations and those made from sputum often show, besides these bipolar forms, great variation in the morphology of the organisms present. Involution forms, consisting of longer, thicker, deeply staining rods, or of organisms which have assumed a spherical or orbicular outline, or, occasionally even appearing very much as yeast cells, may be encountered. Many of these forms stain poorly, or sometimes only a portion of the organism is stained, and in the shorter bacilli the appearance of ring forms is thus produced. In agar-cultures, and particularly in 3 per cent salt agar, these large involution forms and degenerating organisms of very different shapes are very numerous and characteristic: long and slender or thick bacilli and also boat-shaped, dumb-bell, ring-shaped, and spherical organisms may all be observed. The organism generally appears in preparations from agar-cultures as a short or longer rod, and does not so frequently reveal the marked bipolar appearance when stained. In hanging-drop preparations no true motility is exhibited. No flagella are visible in properly-stained preparations, and no spores have been demonstrated. It stains easily with all the anilin dyes, and particularly well with dilute carbol-fuchsin solution, and is easily and completely decolorized by Gram's stain.

CULTURAL CHARACTERISTICS.

The cultural characteristics of the bacillus also are practically identical with those of many bubonic strains. The bacillus grows well upon neutral, or slightly alkaline, moist agar at a temperature of from 25° to 35° C., and is aërobic. After twenty-four to forty-eight hours on agar-cultures inoculated with the pneumonic strain, usually small, delicate, transparent, dewdrop-like colonies appear, which after forty-eight to seventy-two hours have increased in size, are more raised, and have become less translucent. After this time many of these colonies do not perceptibly increase in diameter, while others later become much heavier and larger, so that the two types of colonies are frequently observed in the same culture: the one smaller and more or less translucent, and the other much larger (four or five times the

diameter of the smaller ones) and whiter and more opaque in character. The organism grows more slowly when first transplanted from the animal body to agar than in subsequent transplantations on agar. Microscopically, the colonies exhibit a lighter peripheral band and a thicker, raised, slightly granular center which, when examined by direct light, gives a considerably darker appearance than the periphery.

In gelatin, somewhat similar, though slightly more delicate, colonies to those on agar are formed. In stab-cultures the gelatin is not liquefied. It was pointed out by some observers at the Conference that some of the freshly-isolated pneumonic cultures caused no turbidity when grown in bouillon, the growth rapidly falling to the bottom of the media and leaving the supernatant fluid clear. However, this is not invariably the case, as other pneumonic cultures cause slight turbidity. These variations in the different cultures evidently depended more upon the amount and manner of inoculation of the organisms and the character of the media than upon any particular characteristic of the culture itself.

In Manila, we have, since the Conference, studied three different bubonic cultures recently isolated—one from Shanghai, one from Hongkong, and one from Mariveles, Philippine Islands—and three cultures from different pneumonic cases. These were each inoculated in tubes of bouillon and grown side by side at room temperature. No difference in growth as to the cloudiness of the bouillon, amount of sediment, etc., could be observed in the different tubes. In one of the pneumonic cultures and in one of the bubonic ones the growth and flocculi seemed somewhat heavier than in the other tubes. The growth in all of the cultures became visible, about the second day, in the form of fine flocculent masses which later greatly increased in size and became deposited partly along the sides and at the bottom of the tubes. The bouillon in all was slightly clouded. Microscopical specimens from the different cultures revealed chains of coccoid bacilli.

MUCUS PRODUCTION.

The production of mucus by the pneumonic strain when grown upon agar slants has been marked, but varies greatly, as is also the case with bubonic strains, according to the temperature at which the cultures develop. Grown at ice-box temperature, the culture forms a crumbly mass when collected upon the platinum loop and, when this mass is placed in saline solution, it is ex-

tremely difficult to break up the small clumps and to obtain a homogeneous and durable suspension. When grown at 37° C., the bacilli, when collected upon the platinum loop, form a homogeneous, moist, mucoid mass which readily forms a homogeneous suspension when shaken in saline solution. At 30° C., the growth results sometimes more like the growth at 37° C. just described, at other times more like the growth at the temperature of the ice-box, depending upon the strain employed; most strains cultivated at 30° C. produce more mucus than is usually seen in cultures developed in the ice-box and less than is seen in cultures grown at 37° C.

Another factor, which in our experience has exerted an important influence upon the mucus production of a plague strain, is the length of time it has been cultivated upon artificial media. Freshly-isolated strains, whether from human subjects or from our experimental animals, produce more mucus than strains which have been cultivated on agar for some time.

We cultivated a number of strains at 32° C. upon sugar-free agar, glucose-agar, saccharose-agar, and starch-agar, and did not observe that these carbohydrates caused an increase in the mucus production.

The age of the culture is a factor influencing the amount of mucus present. A twenty-four-hour culture will contain less mucus than the same culture several days later.

We have pointed out that the readiness with which the strains form homogeneous suspensions appears to run parallel with their mucus production and hence the former serves as a good index of the latter. There are, however, other factors which bring about the formation of homogeneous suspensions, notably the presence of alkali. The addition of a few drops of alkali to a suspension of a culture grown at ice-box temperature and shaking quickly brings about the disappearance of the clumps, and a homogeneous suspension results. It is for this reason difficult to determine whether or not the reaction of the culture-medium exerts an influence upon the mucus-production of plague organisms; we can only affirm that this influence, if present, is not marked.

To sum up, in our opinion the two factors of paramount importance with regard to the mucus production of plague bacilli are the temperature at which the cultures are grown and the length of time that the organisms have been cultivated on artificial media since their isolation from the animal host.

Bearing these facts in mind, we have not observed with regard

to mucus production that our pneumonic-plague strains in any way differ from the bubonic strains.

VIRULENCE.

The organism seems to have retained a maximum virulence throughout the epidemic, at least all of the cultures isolated and studied by inoculation into animals possess this very high degree of virulence. Cultures isolated near the close of the epidemic showed an equally high virulence to those isolated near its beginning. However, the idea that this epidemic of pneumonic plague was due to the fact that the strain possessed an abnormally high virulence—much greater than that possessed by the organism of bubonic plague—and that this accounted for the very high mortality during the epidemic appears to be erroneous. The very acute course of the disease, the very high death rate during the epidemic as compared with that of bubonic plague, and the apparently increased virulence of this pneumonic strain may be satisfactorily explained by the fact that the portal of entry of the organism and the location of the primary points of infection in pneumonic plague and in bubonic plague are different. The plague organism finds in the pulmonary tissues a much more favorable and extensive medium for its multiplication and diffusion than it does in the lymphatic glands. In bubonic plague, the lymphatic glands may be said to act as filters against the general invasion of the organism by the plague bacillus, while in primary pneumonic plague there is no such mechanism for the defense of the host, the bacilli spreading rapidly throughout the lung and invading the circulation in every instance in a comparatively short time and apparently before the organism has had time to produce any appreciable quantity of immune substances. The bronchial lymphatic glands in primary pneumonic plague offer resistance to the invasion of the plague bacillus, and in every case of this disease these glands are very acutely inflamed and frequently almost of a black color from the resulting toxic hæmorrhages in the glandular substance. However, by the time the bronchial glands have become involved, the bacteria have already spread so extensively throughout the lung substance that a bacteræmia has usually occurred. Microscopical preparations made at necropsy from the lungs of these pneumonic cases invariably contain enormous numbers of plague bacilli. In no other disease are the organisms found in such great abundance. In primary pneumonic plague, the bacilli are found in very much greater number in the lung than in the spleen,

even though an advanced bacteræmia is present. This fact, also, suggests that the lung tissue offers a more favorable location for the growth and multiplication of the bacilli than does the spleen. The bacteria are also present in far greater numbers in the lung than they are ever found in the buboes or spleen in bubonic-plague cases. It is, also, evident that in pneumonic plague the infected lung (which may be said to correspond to the primary bubo of bubonic plague) contains, by reason of the size of the infected area, a far greater number of plague bacilli than the primary bubo in bubonic plague. During epidemics of bubonic plague, there are occasionally small epidemics of pneumonic plague in which the same high mortality and acute course of the disease is observed as occurred in the Manchurian epidemic of pneumonic plague. This is another argument in favor of the fact that during epidemics of bubonic plague the causative organism may show the same high virulence. As examples may be cited the epidemic of bubonic plague in Japan—in Kobe and in Osaka in 1899 to 1900—in which 13 cases of primary pest pneumonia all terminated fatally after a very rapid course, and the epidemic of bubonic plague in 1898 in Bombay in which, toward its close, 11 cases of pneumonic plague also all quickly succumbed one after the other.

All this evidence is in favor of the supposition that the organism giving rise to the present epidemic is of no greater virulence than in the case of many bubonic strains; furthermore, definite proof of this fact has been obtained from comparative inoculations made in animals with different pneumonic and bubonic cultures. Many of our experiments have been reported in the testimony of the Conference, and will not be given in detail here;[1] the results of others performed by us since that time are recorded in Table I.

The guinea pigs were all inoculated in the following manner: An area of the abdomen, about 2 centimeters square, was shaved and scraped with the razor until petechial hæmorrhages appeared in the skin. A 42-hour agar-slant-culture of each organism was suspended in 5 cubic centimeters of peptone solution and 5 *œsen* of each suspension were rubbed over the shaved area of the guinea pig's abdomen. At necropsy the animals showed the usual lesions of bubonic plague. These guinea pigs were inoculated on June 8. The pneumonic strains were isolated during the month of March, the bubonic strain "Hongkong" on May 20, and the bubonic strain "Mariveles" on May 27. The bubonic strain sent from Shanghai had been on artificial media at least for several months.

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 75, and Index under Virulence.

TABLE I.—*Showing virulence of various cultures of plague.*

Number of guinea pig.	Weight of guinea pig.	Number of culture.	Number of days before death.
	Grams.		*Days.*
5258	400	1	3½
5259	390	2	4
5261	350	5	6¼
5262	360	7	7¼
5263	340	8	7
5264	400	9	3
5265	360	10	7
5266	350	21	6
5267	390	22	5½
5292	350	16	8
5293	380	23	6½
5294	390	25	6
5295	390	26	5
5297	330	28	3
5298	350	29	5½
5300	370	31	5½
5325	330	11	5
5326	350	12	7
5327	380	13	5½
5328	360	14	10
5329	380	15	5½
5330	360	17	5
5331	380	18	4½
5332	370	19	5
5334	350	Shanghai.	(a)
5335	360	Mariveles.	5½
5336	340	Hongkong.	5
5415	340	32	9

[a] Developed buboes, but recovered.

These experiments and those already referred to (*loc. cit.*) have shown that the pneumonic cultures have not possessed any greater virulence than that possessed by many virulent bubonic ones of the organism. Mice, rats, guinea pigs, and monkeys inoculated with virulent bubonic cultures die within the same period of time and from the same doses as do the corresponding animals inoculated with the pneumonic cultures. The same lesions are observed in animals after inoculation of the pneumonic strain as after the inoculation of the bubonic strain. Both strains when inoculated cutaneously, or subcutaneously, into guinea pigs and monkeys give rise to bubonic-plague infection. When the animals are infected by inhalation with either strain, similar lesions are also produced. In guinea pigs, after inhalation, infection results through the mucous membrane of the throat and upper portion of the respiratory tract, resulting in buboes of the cervical glands and septicæmia and in primary or secondary pneumonia; in monkeys, after infection by inhalation, primary pneumonic infection of the lung with secondary septicæmia results.

However, while during epidemics of bubonic plague reports

have been made that there is often a marked difference in virulence in the different cultures isolated, during this epidemic of pneumonic plague the organism seems to have retained a very high degree of virulence throughout. The cultures isolated from a number of cases near the close of the epidemic, upon inoculation into animals, proved to be fully as virulent and to kill animals as quickly and in the same doses as did those cultures isolated near the beginning. That the organism retained such a stable virulence throughout the epidemic is, perhaps, not surprising when one considers that infection occurred directly from man to man or, frequently one might say, from lung to lung and without the passage of the organism through rodents as ordinarily occurs in bubonic-plague infection. Morever, from the results of previous experiments relating to infection of animals with pneumonic plague by inhalation, we would expect that the organism would have retained its maximum virulence throughout this epidemic.

For these reasons and, also, from the fact that the acute course and mortality of the disease were not changed toward the close of the epidemic and especially, from the experimental proof furnished by the inoculation of animals with cultures isolated near the beginning and near the close of the epidemic, we must conclude that the sudden decline and cessation of the epidemic was not due to any marked change in the virulence of the strain. Such a decline and cessation must have depended upon other causes. The plague bacillus, whether isolated from pneumonic or from bubonic epidemics, usually exhibits marked stability in virulence. While it is not a very resistant organism in nature and easily becomes destroyed under certain conditions, it usually does not become markedly attenuated in passage through the animal body, and even on artificial culture-media, after many months, its virulence is usually fully retained. Instances of spontaneous loss of virulence in culture-media have been reported, but this is not usually the case with fresh, virulent cultures. This quality of stability of virulence of the plague bacillus, so different, for example, from that of the cholera vibrio, is of particular interest from an epidemiological standpoint.

AGGLUTINATION TESTS.

Theoretically the agglutination test has two applications in plague: (1) The diagnosis of the disease by the demonstration of antibodies in the patient's serum and (2) the identification of the organism cultivated from a suspected case by means of the serum of an animal immunized against the plague bacillus.

In pneumonic plague, the agglutination test has no clinical value, for the patients succumb to the disease before antibodies are produced or at least produced in any quantities that are capable of detection.

With regard to the second application of the method, as there seemed to be some difference of opinion as to the value of the agglutination test in identifying plague bacilli, we decided, after our return to Manila, to carry out a series of experiments in the hope of throwing further light upon this subject. The result of our experiments in this direction will be described briefly.

For obtaining an agglutinating serum, rabbits were used and large doses of the bacilli were injected intravenously. Repeated inoculation of *living avirulent* plague bacilli administered in this way called forth a very satisfactory serum, but *killed virulent* bacilli failed to do so in every instance. All of the rabbits lost weight rapidly during immunization.

After obtaining a satisfactory serum, the various pneumonic strains which we had brought back with us from Mukden, together with three bubonic strains and three strains from experimental animals, were all subjected to the agglutination test with the same plague serum. The organisms were grown at 32° C. In order to avoid spontaneous sedimentation, the bacteria were suspended in distilled water and the dilutions of the sera made with 0.1 per cent sodium chloride solution.

The greatest dilution of the immune serum, which caused complete or almost complete agglutination, is recorded for each strain tested in the following table:

TABLE II.—*Showing limit of agglutination in plague strains.*

Strain.	Limit of agglutination.	Strain.	Limit of agglutination.
Pneumonic plague:		Pneumonic plague—Continued.	
No. 1	160	No. 22	1,280
No. 5	160	No. 23	1,280
No. 7	640	No. 25	320
No. 8	320	No. 26	320
No. 9	160	No. 28	320
No. 10	160	No. 29	320
No. 12	640	No. 32	320
No. 13	160	Shanghai	320
No. 14	640	Hongkong	640
No. 15	320	Mariveles	320
No. 16	160	Avirulent plague	(?)
No. 17	320	Guinea pig:	
No. 18	640	No. 5635	80
No. 19	640	No. 5769	80
No. 21	640	No. 5745	80

Two points are strikingly obvious from this series of experiments: (1) There is great variability in the limits of agglutination of the different strains and (2) the strains freshly isolated from experimental animals agglutinate only at relatively low dilutions of the serum. It is also to be noted that both pneumonic strains and bubonic ones are agglutinated by the same serum.

It was next decided to select certain of these strains in order to make a careful study of the influence of various factors upon their agglutinability.

One of the difficulties in performing agglutination tests, particularly with strains which have been grown on artificial media for long periods of time, has been the tendency of the bacilli to form clumps spontaneously. We found that very small amounts of alkali would prevent this spontaneous flocculation and sedimentation of the suspensions, but on performing tests with such suspensions these extremely small amounts of alkali inhibited all agglutination.

It has already been mentioned that we performed a series of tests in the presence of only a small amount of electrolytes (0.1 per cent sodium chloride instead of 0.8 per cent). This served well to render the suspensions homogeneous and durable and agglutination was not inhibited, but there was frequently a disturbing flocculation with normal serum within very narrow limits, such as is often seen when one colloid is flocculated by another. As only two or three tubes of a long series were so affected, it was possible to distinguish between this phenomenon and the true specific agglutination. Nevertheless, the method was abandoned and in the tests to be described below both the bacterial suspensions and the dilutions of the serum were prepared with 0.5 per cent sodium chloride solution.

The following tables, selected from a number of similar ones with other strains of plague bacilli, demonstrate clearly the results of our experiments.

TABLE III.—*Showing agglutination of culture No. 26.*

	Grown at 37° C.			Grown at 32° C.			From guinea pig. Grown at 37° C.			From guinea pig. Grown at 32° C.			From guinea pig. Grown at 12° C.		
	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	3 hours.	20 hours.
Serum of immunized rabbit:															
1/20	++	+++	+++	+	++	+++	++	+++	+++	+	++	+++	trace	+	+++
1/40	++	+++	+++	+	++	+++	++	+++	+++	+	++	+++	trace	+	+++
1/80	+	+++	+++	+	++	+++	—	—	+	+	++	+++	trace	+	+++
1/160	—	—	trace	+	++	+++	—	—	—	+	++	+++	trace	+	+++
1/320	—	—	trace	+	++	+++	—	—	—	+	++	+++	trace	+	+++
1/640	—	—	—	+	++	+++	—	—	—	trace	++	+++	trace	+	+++
1/1280	—	—	—	+	++	+++	—	—	—	—	trace	++	trace	+	+++
1/2560	—	—	—	trace	++	+++	—	—	—	—	trace	++	trace	+	+++
1/5120	—	—	—	trace	+	+++	—	—	—	—	trace	++	trace	+	+++
1/10240	—	—	—	—	+	+++	—	—	—	—	trace	+	—	+	+++
Salt solution	—	—	—	—	trace	+++	—	—	—	—	trace	+	—	+	+++
Normal rabbit serum:															
1/20	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/40	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/80	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/160	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/320	—	—	—	—	trace	+++	—	—	—	—	—	trace	—	+	+++
1/640	—	—	—	—	trace	+++	—	—	—	—	—	trace	—	+	+++
1/1280	—	—	—	—	trace	+++	—	—	—	—	—	trace	—	+	+++
1/2560	—	—	—	—	trace	+++	—	—	—	—	trace	+	—	+	+++
Salt solution	—	—	—	—	trace	+++	—	—	—	—	trace	+	—	+	+++

TABLE IV.—*Showing agglutination of culture No. 12.*

	Grown at 37° C.			Grown at 32° C.			From guinea pig. Grown at 37° C.			From guinea pig. Grown at 32° C.			From guinea pig. Grown at 12° C.		
	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.
Serum of immunized rabbit:															
1/20	++	+++	+++	++	++	+++	++	+++	+++	+	++	+++	+	+	+++
1/40	++	+++	+++	++	++	+++	++	+++	+++	+	++	+++	+	+	+++
1/80	++	+++	+++	++	++	+++	—	+	++	+	++	+++	+	+	+++
1/160	trace	+	++	++	++	+++	—	—	—	trace	++	+++	+	+	+++
1/320	trace	+	++	+	++	+++	—	—	—	trace	+	+++	+	+	+++
1/640	trace	+	++	+	++	+++	—	—	—	—	trace	++	+	+	++
1/1280	trace	+	+	+	++	+++	—	—	—	—	—	trace	+	+	++
1/2560	trace	+	+	+	++	+++	—	—	—	—	—	trace	+	+	++
1/5120	—	—	—	+	+	++	—	—	—	—	—	trace	trace	+	++
1/10240	—	—	—	+	+	+++	—	—	—	—	—	trace	trace	+	+++
Salt solution	—	—	—	—	trace	++	—	—	—	—	—	trace	trace	+	+++
Serum of normal rabbit:															
1/20	—	—	—	trace	trace	+	—	—	—	—	—	—	trace	+	+++
1/40	—	—	—	trace	trace	+	—	—	—	—	—	—	trace	+	+++
1/80	—	—	—	trace	trace	+	—	—	—	—	—	—	trace	+	+++
1/160	—	—	—	trace	trace	++	—	—	—	—	—	—	trace	+	+++
1/320	—	—	—	+	+	++	—	—	—	—	—	trace	trace	+	+++
1/640	—	—	—	+	+	++	—	—	—	—	—	trace	trace	+	+++
1/1280	—	—	—	trace	trace	++	—	—	—	—	—	trace	trace	+	+++
1/2560	—	—	—	—	trace	++	—	—	—	—	—	trace	trace	+	+++
Salt solution	—	—	—	—	trace	++	—	—	—	—	—	trace	trace	+	+++

TABLE V.—*Showing agglutination of culture "Hongkong."*

	Grown at 37° C.			Grown at 32° C.		
	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.
Serum of immunized rabbit:						
1/20	++	++	+++	+	+	+++
1/40	++	++	+++	+	+	+++
1/80	+	++	+++	+	+	+++
1/160	+	++	+++	+	+	+++
1/320	+	++	+++	+	+	+++
1/640	+	++	+++	+	+	+++
1/1280	+	++	+++	+	+	+++
1/2560	+	+	++	+	+	+++
1/5120	trace	+	++	+	+	+++
1/10240	trace	+	++	+	+	+++
Salt solution	—	+	+	+	+	+++
Serum of normal rabbit:						
1/20	—	—	—	—	trace	++
1/40	—	—	—	—	trace	++
1/80	—	—	—	—	+	++
1/160	—	—	+	—	+	+++
1/320	—	—	+	—	+	+++
1/640	—	+	+	+	+	+++
1/1280	—	+	+	+	+	+++
1/2560	—	+	+	+	+	+++
Salt solution	—	+	+	+	+	+++

In Table III it is seen that the strain used agglutinates at greater dilutions when grown at 32° C. than when grown at 37° C. The control tubes with normal serum show in the case of the bacteria grown at 37° C. no agglutination whatsoever, even after twenty hours, while in the cases of the bacilli grown at 32° C., the bacteria have all settled out in twenty hours.

The same strain, after passage through an animal, does not agglutinate at as great dilutions as before. When grown at 32° C., it also shows less tendency toward spontaneous sedimentation than previous to the passage through the guinea pig. When grown at 12° C., flocculation begins almost as soon in the tubes with normal serum as it does in those containing immune serum, so that it is difficult to determine whether or not specific agglutination has taken place; however, after twenty hours, on shaking the tubes, the sediment in those with normal serum readily forms a homogeneous suspension, while in the first few tubes, at any rate of those containing immune serum, the sediment is seen to consist of coarser flocculi.

These same observations apply in a general way to the strain used in Table IV. This strain, however, when cultivated at

37°C. agglutinates at much greater dilutions of the same immune serum than does the previous strain when grown at this same temperature. Furthermore, the differences in agglutinability before and after passage through an animal are more marked.

The strain of Table V, when grown at 37°C., agglutinates at still greater dilutions. When cultivated at 32° C., flocculation takes place almost as quickly with normal serum as with immune serum, and it is difficult to decide whether or not specific agglutination has taken place.

Our strain of *avirulent plague* may be cited as the extreme of this series, showing varying grades of agglutinability. Even when cultivated at 37°C., spontaneous sedimentation takes place so rapidly that it is impossible to say that specific agglutination has occurred. Although this strain was used in producing the immune serum, we have in no single instance been able to show that it was agglutinated specifically by the serum.

The same immune serum was used for the tests recorded in Tables III, IV, and V.

We have previously noted that the plague bacillus forms more mucus when cultivated at 37°C. than when grown at 32°C. or even lower temperatures and that more homogeneous and more durable suspensions result in the former instance. We now see that the bacillus when grown at 37°C. is agglutinated with greater difficulty by a specific serum. It, therefore, seems not unlikely that the decrease in agglutinability is due to the increase in mucus production. In harmony with this view is also the fact that strains freshly isolated from experimental animals produce more mucus and are less readily agglutinated by a specific serum than are strains which have been cultivated for months upon artificial media, though the factor of possible difference in virulence also must be considered in this instance.[2]

Finally, we may add that during the course of these experiments we have been able to identify promptly by the agglutination test two strains which were isolated from bubonic cases of plague dying upon ships in the harbor of Manila.

While one of the difficulties in the performance of the agglutination test with the plague bacillus is the tendency toward spontaneous flocculation, we are inclined to believe that, under proper conditions, spontaneous flocculation usually does not occur in freshly-isolated strains; in most strains which have been grown upon artificial media for long periods of time it can be avoided by

[2] Strong, *Journ. Exp. Med.* (1905), 7, 229.

cultivating them at 37°C. The greater difficulty sometimes is, in our opinion, to obtain a satisfactory immune serum. We gave several rabbits repeated intravenous injections of large doses of killed virulent cultures without obtaining more than a trace of agglutination with their sera. We can strongly recommend the use of the living avirulent culture for the preparation of the immune serum.

In conclusion, we can only warn against the use of cultures grown at ice-box temperature as recommended by Shibayama[3] for the agglutination tests. Although such cultures are readily agglutinable, flocculation in the control tubes is apt to be very confusing. If one has a satisfactory immune serum, the culture grown at 32°C. or even at 37°C. will be agglutinated promptly and the control tubes will remain practically unchanged. Controls with normal serum should always be prepared in performing the test.

INFECTIVITY OF THE EXCRETA.

In no other disease is the infecting organism found in such abundance in the sputum as it is in pneumonic plague. When the disease is well developed, *Bacillus pestis* is present in almost pure culture. In pneumonic plague as in bubonic plague, when the disease becomes septicæmic, the organisms are sometimes found in the urine and even sometimes in the fæces. When once the sputum of pneumonic-plague cases becomes thoroughly dried it is no longer infectious, but when the sputum becomes frozen and pulverized, particles of it may be blown about and remain infective for long periods of time or until the sputum is again thawed.

BACTERIOLOGICAL DIAGNOSIS OF PNEUMONIC PLAGUE.

EXAMINATION OF THE SPUTUM.

A bacteriological diagnosis from the sputum can not be made at the onset of the disease, and not until after the fever has developed does the sputum appear. Shortly after the appearance of the sputum, the plague-organism, even if not visible from the microscopical examination, may be isolated by culture. When the sputum becomes bloody, the organism is usually present in large numbers and in almost pure culture. Sometimes the organism might be mistaken morphologically for *Diplococcus pneumoniæ*, and bipolar-staining organisms, other than plague bacilli, may

[3] *Centralbl. f. Bakt. etc.*, Orig. (1905), 1 Abt. 38, 482.

sometimes be encountered in the sputum. While in the microscopical examination of the sputum Gram's stain is a very valuable aid in arriving at a diagnosis of the organism, nevertheless, Gram-negative bacilli have been encountered in the sputum, which proved later not to be plague bacilli. However, usually if the sputum is blood-stained, from the microscopical examination, with the aid of Gram's stain, there is no difficulty in arriving at a diagnosis, since the plague organism is usually present in such very large numbers. In the later stages of the disease, involution forms are commonly encountered in the sputum. The organisms are constantly found in great abundance up to the time of death.

BACTERIOLOGICAL EXAMINATION OF THE BLOOD.

In the early stages of the disease, cultures from the blood are frequently negative. Sometimes, however, the organism could be cultivated from the blood from twenty-four to forty-eight hours before death, and it could always be obtained from the blood a few hours before death. In many instances the bacteria are present in very large numbers in the blood, so that a diagnosis can often be made from a simple, microscopical examination. In no other disease is so marked a bacteræmia present. In the early stages of the disease, cultures from the blood should be made in bouillon, as much as 1 cubic centimeter of blood being employed. The agglutination test is of no value in making a diagnosis, as the course of the disease is too acute and the patient has succumbed before the agglutinins appear in demonstrable quantities. The reaction of the deflection of the complement is also not to be recommended for the same reason; the examination of the sputum and blood for the presence of the bacillus gives much greater and more valuable information. In cases where no necropsy is permitted and a post-mortem bacteriological diagnosis is advisable, microscopical examination of material, obtained by lung puncture with a syringe, may often be conclusive of pneumonic plague, *Bacillus pestis* being present in the microscopical preparation, in enormous numbers, in pneumonic-plague cases.